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Bommineni Harish
P.G Scholar, Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science, P.V. Narasimha Rao Telangana Veterinary University, Rajendranagar, Hyderabad, 500030, Telangana, India

Pabbathi Shivakumar
Assistant Professor, Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science, P.V. Narasimha Rao Telangana Veterinary University, Rajendranagar, Hyderabad, 500030, Telangana, India

Bharani Kala Kumar
Assistant Professor, Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science, P.V. Narasimha Rao Telangana Veterinary University, Rajendranagar, Hyderabad, 500030, Telangana, India

Boinapally Ramya
Assistant Professor, Veterinary Clinical Complex, College of Veterinary Science, P.V. Narasimha Rao Telangana Veterinary University, Rajendranagar, Hyderabad, 500030, Telangana, India

Nisaath Begum
P.G Scholar, Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science, P.V. Narasimha Rao Telangana Veterinary University, Rajendranagar, Hyderabad, 500030, Telangana, India

Matukumalli Usha Rani
Professor and Head, Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science, P.V. Narasimha Rao Telangana Veterinary University, Rajendranagar, Hyderabad, 500030, Telangana, India

Banothu Anil Kumar
Assistant Professor, Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science, P.V. Narasimha Rao Telangana Veterinary University, Rajendranagar, Hyderabad, 500030, Telangana, India

Correspondence:
Dr. Nisaath Begum
P.G Scholar, Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science, P.V. Narasimha Rao Telangana Veterinary University, Rajendranagar, Hyderabad, 500030, Telangana, India
Email: nisaath21@gmail.com

Amelioration of experimental nephrotoxicity due to 5-Flourouracil by Resveratrol in comparison to Vitamin-E

Bommineni Harish, Pabbathi Shivakumar, Bharani Kala Kumar, Boinapally Ramya, Nisaath Begum*, Matukumalli Usha Rani, Banothu Anil Kumar

ABSTRACT

The therapeutic efficacy of Resveratrol (RSV) and Vitamin E were studied against 5-Flourouracil (5-FU) induced nephrotoxicity. 36 male Wistar rats were selected randomly weighing between 150-180 g and are made into 6 groups, each group containing 6 rats. Group 1 was maintained as sham. 5-Flourouracil was administered to groups 2, 5 and 6 intraperitoneally (20 mg/kg body weight) on day 1, 3 and 7. Group 2 was kept as positive control (administered 5-FU intraperitoneally). Groups 3, 5 and 4, 6 were administered vitamin E and resveratrol per orally for 14 days @ 200 mg/kg bwt. At the end of the experiment the blood was withdrawn and serum analyzed for renal biomarkers. For histopathological studies, samples of kidney tissue collected by inducing euthanasia in rats. The sero-biochemical analysis revealed a significant increase in BUN and creatinine values of the rats in group 2. The antioxidant activity was analyzed and the rats in group 2 revealed a significant rise in the values of protein carbonyl, TBARS and significant decrease in GSH. Group 2 also showed an increase in TNF-α and decrease in interleukin-10 concentration. Sections of kidney tissue collected from group 2 showed marked dilation and elongation of tubules, moderate infiltration with inflammatory cells degeneration of bowman's capsule and tubular congestion. Comparatively, groups undergone treatment showed amelioration in the parameters. Thus, resveratrol and vitamin-E exert protective actions against 5-flourouracil (5-FU) induced nephrotoxicity.

Keywords: 5-Flourouracil, Resveratrol, Vitamin-E, Nephrotoxicity.

INTRODUCTION

5-FU is a pyrimidine antimetabolite, one of the broadspectrum anticancer drug used for the treatment of malignancies like glioblastoma, breast cancer, pancreas, ovary, solid cancer such as stomach, colon and lung [1]. 5-FU exerts its action by altering the nucleoside metabolism and inhibit synthesis of deoxyribonucleic acid (DNA) and incorporated into DNA and RNA, leading to cytotoxicity. Various side effects are associated with the 5-FU treatment such as mucositis, stomatitis, vomiting, myelosuppression, leukopenia [2], cardiotoxicity [3], hepatotoxicity and nephrotoxicity [4].

Resveratrol (RSV) is a naturally-occurring polyphenol that is formed in various plants, grapes, berries, peanuts and in red wine. Resveratrol acts as a powerful antioxidant, oxygen free radical scavenger and induces protective enzymes such as heme oxygenase [5,6]. Antioxidant efficacy of resveratrol has been demonstrated in gentamicin-induced nephrotoxicity [7].

Vitamin E have antioxidant properties, protects tissues and counters the detrimental consequences of free radical damage. Vitamin E is the main peroxyl radical scavenger and it maintains the integrity of long-chain poly unsaturated fatty acids (PUFA) in the membranes of cells and thus maintains their bioactivity [8]. Objectives of investigation were to study the nephrotoxicity due to 5-FU and to evaluate and compare nephroprotective potential of resveratrol with vitamin E.

MATERIALS AND METHODS

Reagents

Reagents used in the experimental procedure were of analytical grade, procured from SRL Pvt. Ltd., Mumbai and Qualigens Pvt. Ltd., Mumbai, India.

Experimental animals

36 male Wistar rats weighing between 150-180 g (3 months age) were procured from Vyas labs, Hyderabad. Polypropylene cages were used for housing the animals and proper hygienic conditions were maintained at temperature (20–22^{0}C) and twelve-hour dark and light cycles throughout the experimental

procedure. Animals were kept for acclimatization for a period of seven days. Commercial standard pellet feed and water *ad libitum* provided to the animals throughout the experimental procedure. All the protocols implemented throughout the experimental period were in congruent with the guidelines of Institutional Animal Ethics Committee (No.3/22/C.V.Sc., Hyd. IAEC- Rats/29.02.2020). A total of 36 male *Wistar* rats were divided into 6 groups, comprising of 6 rats in each group and were treated accordingly as shown in Table 1.

Table 1: Design of experiment

Group	Treatment	No. of animals
1	Normal saline	6
2	5-FU (20 mg / kg body weight intraperitoneally) on days 1, 3 and 7	6
3	Resveratrol (200 mg/kg body weight Per oral) for 14 days (200 mg/kg body weight Per oral)	6
4	Vitamin E administered for 14 days (200 mg/kg body weight Per oral)	6
5	5-Flourouracil on days 1, 3 and 7 (20 mg/kg body weight intraperitoneally) + Resveratrol for 14 days (200 mg/kg bodyweight Per oral)	6
6	5-Flourouracil on days 1, 3 and 7 (20 mg/kg body weight intraperitoneally) + Vitamin E for 14 days (200 mg/kg body weight Per oral)	6

After performing the experimental procedures, feed was withdrawn for 12 hours prior the blood collection for sero-biochemical analysis. Blood withdrawn from retro-orbital plexus of animals into vacutainers, the collected blood was centrifuged for 15 minutes at 3000 RPM for segregation of serum and stored at a temperature of -80^{0}C up to further analysis. The segregated sera samples were analyzed for BUN and creatinine. Euthanasia was performed by disclosure of carbon dioxide and kidney tissue samples were collected and preserved at temperature of -80^{0}C until further analysis of protein carbonyls, GSH and TBARS. For histopathological studies sections of kidney tissue were taken and transferred to fixative (10% of formalin).

BIOCHEMICAL PARAMETERS

Estimation of antioxidant activity

Moron *et al*. (1979) [9] method had been utilized for the analysis of GSH. Malondialdehyde (MDA) was assayed by the methodology of Balasubramanian *et al*. (1988) [10]. Levine *et al*. prescribed method had been employed for the analysis of protein carbonyls (1990) [11].

Estimation of serum BUN and Creatinine

Serum BUN and creatinine was estimated by kits provided by ERBA diagnostics Ltd, Surat, India.

Estimation of Cytokine profile

TNF - *alpha* and IL-10 concentration was estimated by the ELISA kit was procured from Krishgen Bio systems, Mumbai.

Histology

After fixation the kidney tissue samples were subjected to dehydration and then kept for clearance in xylol and paraffin fixed at temperature 55-56°C. Thin sections (5micron thickness) of paraffin blocks were made with microtome.

Statistical analysis

SPSS; version 21 was utilized for statistical analysis (One-way ANOVA). Duncan's multiple comparison tests as post hoc analysis is used for testing the difference between means at level of significance $P < 0.05$.

RESULTS AND DISCUSSION

The concentration of serum BUN (mg/dl) in group 2 (53.41 ± 4.78) was significantly ($p<0.05$) higher contrast to group 1 (30.41 ± 2.98), whereas treatment groups 5 (43.15 ± 2.39) and 6 (43.75 ± 2.61) showed lower values in contrast to group 2 at a level of significance ($p<0.05$) as shown in Table 2. The values corresponding to the sero-biochemical parameters of groups 3 and 4 were analogous to group 1. 5-FU results in rise in values of BUN due to hastened protein catabolism and oxidative damage due to ROS trigger lipid peroxidation resulting in injury to lipids, DNA and proteins in the kidney tissue and also modify the lipid-lipid interactions, membrane fluidity and membrane permeability in the kidney. The values of the estimated serum creatinine concentration (mg/dl) in group 2 (1.35 ± 0.03) was higher in contrast to group 1 (0.65 ± 0.04) at level of significance ($p<0.05$), whereas treatment groups 5 (0.97 ± 0.04) and 6 (0.93 ± 0.04) relatively lesser values in contrast to group 2 at level of significance ($p<0.05$) as shown in table below (Table 2), whereas the values of groups 3 and 4 were analogous to group 1 Due to glomerular dysfunction and renal tubular damage there is rise in serum creatinine and this findings were in concurrent with the studies conducted by Gelen *et al.* (2018) [4]. These results were further substantiated by significant histopathological changes include dilation and elongation of tubules, infiltration with inflammatory cells, degeneration of bowman's capsule, atrophied glomeruli, tubular degenerations. Co-administration of 5-FU with Resveratrol and Vitamin-E could significantly reverse the alterations reported in the serum BUN and creatinine which are contrast to the group 2.The estimation of TBARS values showed higher concentration in group 2 (5.32 ± 0.11) contrast to group 1(1.51 ± 0.05) at a level of significance ($p<0.05$), whereas the treated groups, groups 5 (3.78 ± 0.07) and group 6 (3.53 ± 0.06) showed relatively lesser values in contrast to group 2 at a level of significance ($p<0.05$) as depicted in table ad 2. Estimation of protein carbonyls values showed higher concentration in group 2 (1.71 ± 0.07) in contrast to group 1(0.61 ± 0.07) at a level of significance ($p<0.05$), whereas the treated groups, group 5 (0.89 ± 0.07) and group 6 (0.87 ± 0.07) showed relatively lesser values contrast to group 2 at level of significance ($p<0.05$). The estimated concentration of protein carbonyls in group 3 and 4 are analogous to group 1 as shown in table 2. The estimated values of GSH concentration showed decrease in group 2 (3.22 ± 0.11) in contrast to group 1 (4.51 ± 0.15) at a level of significance ($p<0.05$), whereas the treated groups, group 5 (4.01 ± 0.11) and group 6 (4.04 ± 0.92) showed relatively higher values in contrast to group 2 at a level of significance ($p<0.05$). The concentration of GSH values of groups 3 and 4 were analogous to group 1 as shown in Table 3. These results were concurrent with the study conducted by Adikwu *et al*. (2019) [12]. 5-FU triggers the ROS generation resulting in oxidative stress in the tissues. ROS involved in oxidization of nucleic acids, proteins and lipids. Lipid peroxidation which leads to production of MDA, which is an oxidative stress marker. Resveratrol aids in the termination of LPO and enhances antioxidant activity by stimulation of intracellular antioxidant enzymes [13]. Estimation of TNF- α showed higher values in group 2 (18.93 ± 0.69) in contrast to group 1 (8.11 ± 0.73) at a level of significance ($p<0.05$), whereas the treated groups, group 5 (14.51 ± 0.49) and group 6 (14.11 ± 0.49) revealed relatively lesser values contrast to group 2 at a level of significance ($p<0.05$) as shown in

Table 3. Estimation of IL-10 values revealed lesser values in group 2 (49.25 ± 2.4) contrast to group 1 (78.83 ± 1.16) at a level of significance ($p<0.05$), whereas the treated groups, group 5 (68.39 ± 1.18) and 6 (69.27 ± 1.18) revealed higher values in contrast to group 2 at a level of significance ($p<0.05$). The IL-10 values groups 3 and 4 are analogous to group 1 as shown in (Table 3). NF-kβ plays a modulatory role in cellular activities and also in the genes regulating inflammation [14]. NF-kβ when activated under various stress condition relocates into nucleus and modulate the downstream gene expression and thus causing accretion of several proteins such as COX-2 and pro-inflammatory cytokines. 5-FU results in the overexpression of COX-2 [15], causing excessive synthesis of prostaglandins and stimulation matrix metalloproteinases causing disintegration of collagen, epithelial basement membrane, edema formation and leading to tissue injury and thus promoting the production of pro-inflammatory cytokines including TNF-α [16], decrease in the anti-inflammatory cytokines like IL-10 [17].

Table 2: BUN (mg/dl), creatinine (mg/dl), TBARS (nm MDA released/mg protein), Protein carbonyls (nm/mg protein) in different groups of rats

Groups	BUN	Creatinine	TBARS	Pc
Normal control	30.41 ± 2.98 ^{c}	0.65 ± 0.04 ^{c}	1.51 ± 0.05 ^{c}	0.61 ± 0.07 ^{c}
5-FU control	53.41 ± 4.78 ^{a}	1.35 ± 0.03 ^{a}	5.32 ± 0.11 ^{a}	1.71 ± 0.07 ^{a}
RSV	30.71 ± 2.98 ^{c}	0.61 ± 0.04 ^{c}	1.53 ± 0.05 ^{c}	0.63 ± 0.07 ^{c}
Vit-E	30.81 ± 2.98 ^{c}	0.62 ± 0.04 ^{c}	1.36 ± 0.05 ^{c}	0.62 ± 0.06 ^{c}
5-FU + RSV	43.15 ± 2.39 ^{b}	0.97 ± 0.04 ^{b}	3.78 ± 0.07 ^{b}	0.89 ± 0.07 ^{b}
5-FU+ Vit-E	43.75 ± 2.61 ^{b}	0.93 ± 0.04 ^{b}	3.53 ± 0.06 ^{b}	0.87 ± 0.07 ^{b}

Values are estimated as mean ± SE (n=6); one-way analysis of variance (one way ANOVA). Means with different alphabets show significant ($P < 0.05$) difference

Table 2: GSH (nm/mg protein), TNF-alpha (pg/mg tissue) and IL-10 (pg/mg tissue) in different groups of rats

Groups	GSH	TNF- α	IL-10
Normal control	4.51 ± 0.15 ^{a}	08.11 ± 0.73 ^{a}	78.83 ± 1.16 ^{a}
5-FU control	3.22 ± 0.11 ^{c}	18.93 ± 0.69 ^{c}	49.25 ± 2.14 ^{c}
RSV	4.44 ± 0.16 ^{a}	07.51 ± 0.34 ^{a}	78.79 ± 1.18 ^{a}
Vit-E	4.52 ± 0.15 ^{a}	07.11 ± 0.34 ^{a}	78.45 ± 1.18 ^{a}
5-FU + RSV	4.01 ± 0.11 ^{b}	14.51 ± 0.49 ^{b}	68.39 ± 1.18 ^{b}
5-FU+ Vit-E	4.04 ± 0.92 ^{b}	14.11 ± 0.49 ^{b}	69.27 ± 1.18 ^{b}

Values are mean ± SE (n=6); one-way analysis of variance (one way ANOVA). Means with different alphabets show significant ($P < 0.05$) difference

Histopathology

Histopathology studies of the of the kidney tissue under light microscopic examination in non-toxic control rats of group 1 revealed the normal histological structure (Fig. 1). Whereas 5-Flourouracil (5-FU) control rats of group 2 showed severe histological alterations. Marked dilation and elongation of tubules and moderate infiltration with inflammatory cells (Fig.2). Moderate degeneration of bowman's capsule and tubular congestion (Fig.3). Kidney tissue showing vacant glomeruli and moderate tubular epithelial degeneration (Fig.4). The observed results were in correlation with the findings of Rashid *et al.* (2014) [18]. Whereas the treatment groups show Mild degenerative changes (Fig.5) in group 5, Mild inflammation (Fig.6) in group 6. These restorative changes due to Resveratrol and Vitamin-E treatment during the experimental period.

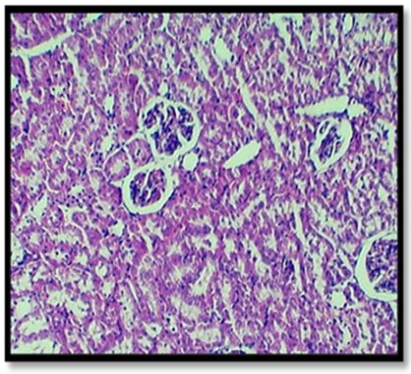

Figure 1: Photograph of microscopic image of kidney tissue illustrating normal architecture of glomeruli and tubules. H&E 100X

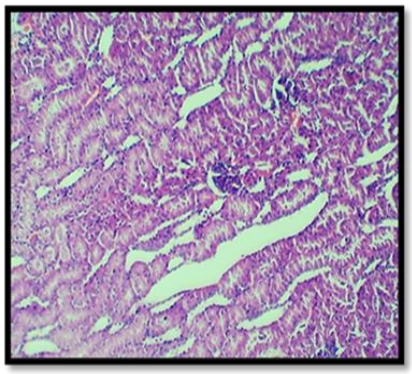

Figure 2: Photograph of microscopic image of kidney tissue illustrating prominent dilation, elongation of tubules (thick arrow) and moderate infiltration with inflammatory cells

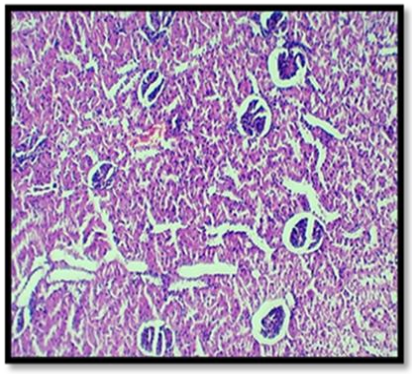

Figure 3: Photomicrograph of Kidney tissue showing moderate degeneration of bowman's capsule (thick arrow) and tubular congestion (thin arrow) H&E 100X

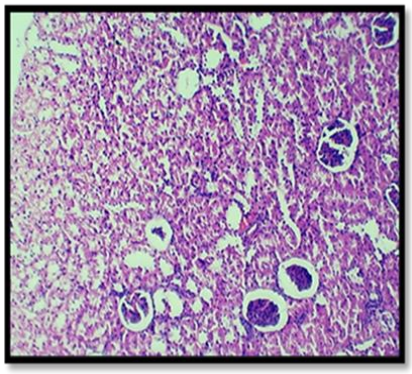

Figure 4: Photograph of microscopic image of kidney tissue illustrating vacant glomeruli (arrow), moderate tubular epithelial degeneration H&E 100X

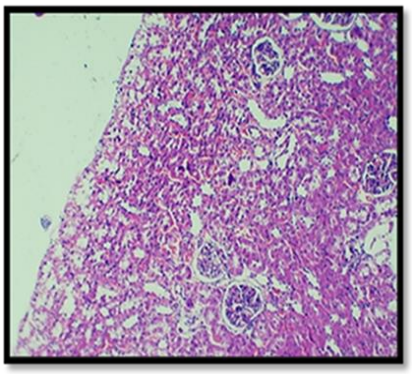

Figure 5: Photograph of microscopic image of kidney tissue illustrating mild degenerative changes (arrow). H&E 100 X

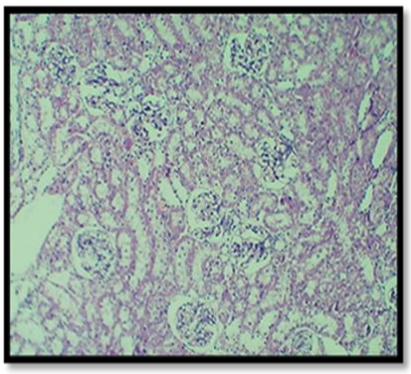

Figure 6: Photograph of microscopic image of kidney tissue illustrating mild inflammation (arrow). H&E 100X

CONCLUSION

Although the plants did not show significant male-driven sex selection activity, when the study was repeated with the administration of similar doses to females, the ratio of males to females in the litter count was significantly increased in all the groups treated with the extracts. This indicates that the plant has the potential to influence the gender of offspring before or during conception in female rats. In conclusion, the findings of our study taken together indicate that high levels of vaginal pH, maternal serum estrogen and cortisol hormone are some of the mechanisms by which *V. amygdalina* causes an increase in sex ratio of offspring in rats. Further studies are recommended to elucidate the mechanisms by which the extracts cause changes in vaginal pH, hormone levels, phytochemicals, and mineral components selectively affect the X- and Y-spermatozoa in aspects of mitochondrial respiration, acrosome integrity, and internal pH leading to increased sex ratios in female rats.

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Conflict of Interest

None declared.

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